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| Phase resolved fluorescence spectroscopy was used to incorporate fluorescence lifetime   |  |  |                               |                |               |  |
| selectivity into mutlicomponent fluorimetric determinations. Quantitative analysis was   |  |  |                               |                |               |  |
| demonstrated for systems of up to six fluorescent components with overlapping spectra.   |  |  |                               |                |               |  |
| Multidimensional data formats of phase∓resolved fluorescence intensity as a function of spectral and lifetime parameters were developed, including formats for both synchronous  |  |  |                               |                |               |  |
| excitation and total luminescence spectroscopy. It was shown that phase resolution improves  |  |  |                               |                |               |  |
| quantitative results significantly over the use of steady state, non-lifetime dependent  |  |  |                               |                |               |  |
| intensity measurements. Specific systems studied include polycylic aromatic hydrocarbons and   |  |  |                               |                |               |  |
| metal chelates. Sodium taurocholate micelles were studied as a means by which synergistic  |  |  |                               |                |               |  |
| interactions between fluorescent molecules in a sample could be minimized. Very interesting  |  |  |                               |                |               |  |
| results were obtained, and further investigations of the micellar systems are being conducted.   |  |  |                               |                |               |  |
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## MULTICOMPONENT ANALYSIS USING SYNCHRONOUS-EXCITATION PHASE-RESOLVED FLUORESCENCE SPECTROSCOPY

FINAL REPORT

SUBMITTED BY LINDA B. McGOWN (P.I.)

DECEMBER 4, 1987

U. S. ARMY RESEARCH OFFICE

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The work described in this report was performed at Oklahoma State University. In the summer of 1987, I left O.S.U. to join the Chemistry Department at Duke University. We are continuing the ARO project here at Duke, under Grant DAA-LO3-87-K-0151.

### A. STATEMENT OF THE PROBLEM STUDIED

We have investigated new ways in which to incorporate fluorescence lifetime selectivity into chemical analysis through the use of phase-resolved fluorescence spectroscopy (PRFS). The approaches that we have taken involve the use of synchronous excitation and total luminescence spectral data formats in which phase resolved fluorescence intensities are collected as a function of the appropriate wavelength parameters, and either detector phase angle or excitation modulation frequency are used to exploit fluorescence lifetime selectivity. The goal is to develop ways in which PRFS could be used for the quantitative and qualitative analysis of real, multicomponent fluoresent samples. The earliest studies focussed on the use of PRFS for quantitative determinations in multicomponent samples, in which the experimental conditions were nonrigorously optimized for the known components in the synthetic mixtures. Later studies involved the development of generalized, multidimensional data formats that could be used for samples in which the identities of the fluorescent components is not known. In the latter studies, data analysis methods such as factor analysis and pattern recognition are being developed to extract maximum information from the spectral data sets.

### B. SUMMARY OF THE MOST IMPORTANT RESULTS

The references given in this section refer to the list of publications in Section C. The numbers refer to chronological order of publication, and are therefore not in sequence in this section.

### 1. Fluorescence lifetime determinations.

The goal of the ARO project is the incorporation of fluorescence lifetime information into fluorimetric analysis. Fluorescence lifetime may be used as a selectivity parameter for the resolution of overlapping spectral contributions. Fluorescence lifetime determinations can also be used directly to provide qualitative information for the identification of components. We have studied several aspects of phase-modulation fluorescence lifetime determinations. In one study, we investigated the effects of various experimental and instrumental factors on the calibration of phase-modulation fluorescence lifetime measurements [4] in order to maximize the accuracy of our lifetime data. In another study, fluorescence lifetimes obtained from phase-resolved fluorescence intensity data were compared with values obtained from direct measurements of phase shift and demodulation [3]. Good agreement was found between values obtained by the two methods. Precision was slightly lower, but still quite good, for the phase-resolved determinations. From this last study, we conclude that it is possible

to determine fluorescence lifetimes from the PRFS data, thereby avoiding the need to run separate phase-modulation experiments.

### 2. Quantitative analysis of multicomponent systems.

Most of our effort in the past project period has been directed towards the evaluation of PRFS for multicomponent determinations. The use of overdetermined sets of linear equations was first applied to a four component system of polycyclic aromatic hydrocarbons (PAHs) that have overlapping fluorescence spectra [1]. A second system of four PAHs was studied, for which two modulation frequencies were used to generate phase-resolved intensity data at various combinations of wavelength and detector phase angle [2]. The experimental conditions used in both studies were optimized for the particular PAHs in the systems.

The next step towards our goal of developing techniques for the analysis of real, complex samples was the development of generalized data formats [5,9] to replace the use of experimental conditions that have been optimized for particular components. This is a necessary step since we would like to be able to analyze samples with minimal knowledge or assumptions regarding their composition. Several data formats were compared, including phase-resolved intensity plotted as a function of detector phase angle on one axis and either emission or synchronously scanned wavelength on the other, and the corresponding steady-state formats with steady-state intensity plotted as a function of wavelength alone. For two- and three-component mixtures, PRFS did not significantly improve the results. However, four-component determinations were best achieved by the PRFS-synchronous excitation data format. This format also gave the best overall performance, which is what we were seeking for a generalized data format. The PRFS-synchronous excitation format was successfully applied to five- and six-component determinations of PAHs [7], and the results were far superior to those obtained with the corresponding steady-state data format. These studies demonstrate the advantages of using PRFS relative to steady-state fluorescence and suggest that accuracy can be increased for multicomponent determinations by the use of multiple modulation We have also demonstrated the use of PRFS for frequencies. two-component determinations of fluorescent metal chelates. These simultaneous determinations cannot be accomplished with steady-state fluorescence alone since the fluorescence spectra of the metals with a given chelating agent are highly overlapping. The PRFS determinations are based solely on fluorescence lifetime differences between the metal chelates of a given chelating agent. Several different chelating agents have been used, including 5-sulfo-8-hydroxyquinoline for mixtures of Al and Ga, In and Ga, and Cd and Zn [8] and lumogallion for mixtures of Al and Ga [11].

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### 3. Suppression of scattered light.

Steady-state data formats such as synchronous excitation spectra and total luminescence spectra generally contain intensity contributions from scattered light. For example, a wavelength difference of 3 nm between the excitation and emission wavelengths is frequently used to obtain synchronous excitation spectra of PAHs. Unfortunately, there is often a large scattered light signal under these conditions since emission is monitored at a wavelength so close to the excitation wavelength. If the spectrum is acquired by measuring the phase-resolved intensity at the detector phase angle required to null the scattered

light contribution, only the emission from the fluorescent components will be observed. We have demonstrated the suppression of scattered light in synchronous spectra of PAHs acquired with a  $\Delta\lambda$  of 3 nm, and showed that detection limits can be improved for some PAHs by the elimination of the large scattered light background signal [6].

We have also demonstrated the suppression of scattered light in total luminescence spectra of crude oil and human serum [10]. The ability of PRFS to suppress the scattered light contributions in highly scattering samples such as those containing proteins, micelles, or high levels of particulate matter, is an important aspect of our current research, in which we are using multiple modulation frequencies to exploit fluorescence lifetime selectivity.

### 4. Studies of sodium taurocholate micelles.

Sodium taurocholate (NaTC) aggregates in water to form four-member micelles over a wide range of experimental conditions. The micelles can bind hydrophobic molecules, generally in a 1:1 micelle:molecule stoichiometry. We are using fluorescent probe molecules to study the structural and binding characteristics of NaTC micelles and NaTC-detergent mixed micelles [13]. We are also very interested in the selective enhancement of PAH fluorescence that we have observed when metal cations are added to NaTC micellar solutions of the PAH compounds [14]. The analytical applications of this work include (1) the selective modification of fluorescence intensity and lifetime properties of fluorescent compounds through association with NaTC micelles and (2) the use of NaTC micelles to simplify the steady-state and PRFS spectra of complex samples by reducing intermolecular interactions (e.g., dimerization, excimer and exciplex formation, excitation transfer, etc.).

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- Nithipatikom, K. and McGown, L. B., "Effects of Metal Cations on the Fluorescence Intensity of Polycyclic Aromatic Hydrocarbons in Sodium Taurocholate Micellar Solutions", submitted to ANAL. CHEM., 10/87.

### D. PARTICIPATING SCIENTIFIC PERSONNEL

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<sup>\*</sup>Ph.D. dissertation will consist entirely of ARO sponsored research.

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